

EXHIBIT B

4,695,623

33

EXAMPLE 8

As discussed infra with respect to consensus leukocyte interferon, those human leukocyte interferon subtypes having a threonine residue at position 14 and a methionine residue at position 16 are reputed to display greater antiviral activity than those subtypes possessing Ala¹⁴ and Ile¹⁶ residues. An analog of human leukocyte interferon subtype F was therefore manufactured by means of microbial expression of a DNA sequence of Example 7 which had been altered to specify threonine and methionine as residues 14 and 16, respectively. More specifically, [Thr¹⁴, Met¹⁶] IFN- α F, designated IFN- α F₂, was expressed in *E. coli* upon transformation with a vector of Example 7 which had been cut with SalI and HindIII and into which a modified subunit II (of Table VII) was inserted. The specific modifications of subunit II involved assembly with segment 39 altered to replace the alanine-specifying codon, GCT, with a threonine-specifying ACT codon and replace the isoleucine-specifying codon, ATT, with an ATG codon. Corresponding changes in complementary bases were made in section 40 of subunit I, e.g., IFN-FII.

The following Examples 9 and 10 relate to practice of the invention in the microbial synthesis of consensus human leukocyte interferon polypeptides which can be designated as analogs of human leukocyte interferon subtype F.

EXAMPLE 9

"Consensus human leukocytic interferon" ("IFN-Con," "LcIFN-Con") as employed herein shall mean a nonnaturally-occurring polypeptide which predominantly includes those amino acid residues which are common to all naturally-occurring human leukocyte interferon subtype sequences and which includes, at one or more of those positions wherein there is no amino acid common to all subtypes, an amino acid which predominantly occurs at that position and in no event includes any amino acid residue which is not extant in that position in at least one naturally-occurring subtype. (For purposes of this definition, subtype A is positionally aligned with other subtypes and thus reveals a "missing" amino acid at position 44.) As so defined, a consensus human leukocyte interferon will ordinarily include all known common amino acid residues of all subtypes. It will be understood that the state of knowledge concerning naturally-occurring subtype sequences is continuously developing. New subtypes may be discovered which may destroy the "commonality" of a particular residue at a particular position. Polypeptides whose structures are predicted on the basis of a later-

amended determination of commonality at one or more positions would remain within the definition because they would nonetheless predominantly include common amino acids and because those amino acids no longer held to be common would nonetheless quite likely represent the predominant amino acid at the given positions. Failure of a polypeptide to include either a common or predominant amino acid at any given position would not remove the molecule from the definition so long as the residue at the position occurred in at least one subtype. Polypeptides lacking one or more internal or terminal residues of consensus human leukocyte interferon or including internal or terminal residues having no counterpart in any subtype would be considered analogs of human consensus leukocyte interferon.

Published predicted amino acid sequences for eight cDNA-derived human leukocyte interferon subtypes were analyzed in the context of the identities of amino acids within the sequence of 166 residues. See, generally, Goedell, et al., *Nature*, 290, pp. 20-26 (1981) comparing LeIFN-A through LeIFN-H and noting that only 79 amino acids appear in identical positions in all eight interferon forms and 99 amino acids appear in identical positions if the B subtype (deduced from a cDNA pseudogene) was ignored. Each of the remaining positions was analyzed for the relative frequency of occurrence of a given amino acid and, where a given amino acid appeared at the same position in at least five of the eight forms, it was designated as the predominant amino acid for that position. A "consensus" polypeptide sequence of 166 amino acids was plotted out and compared back to the eight individual sequences, resulting in the determination that LeIFN-F required few modifications from its "naturally-occurring" form to comply with the consensus sequence.

A program for construction of a manufactured IFN- α Con DNA sequence was developed and is set out below in Table VIII. In the table, an asterisk designates the variations in IFN- α F needed to develop LeIFN-Con, i.e., to develop the [Arg²⁴, Ala⁷⁶, Asp⁷⁸, Glu⁷⁹, Tyr⁸⁶, Tyr⁹⁰, Lys⁹⁶, Thr¹⁵⁶, Asn¹⁵⁷, Leu¹⁵⁸] analog of IFN- α F. The illustrated top strand sequence includes, wherever possible, codons noted to be the subject of preferential expression in *E. coli*. The sequence also includes bases providing recognition sites for Sal, HindIII, and BstEII at positions intermediate the sequence and for XbaI and BamHI at its ends. The latter sites are selected for use in incorporation of the sequence in a pBR322 vector, as was the case with the sequence developed for IFN- α F and its analogs.

TABLE VIII

4,897,471

33

Example 7 which had been altered to specify threonine and methionine as residues 14 and 16, respectively. More specifically, [Thr¹⁴, Met¹⁶] IFN- α F, designated IFN- α F₂, was expressed in *E. coli* upon transformation with a vector of Example 7 which had been cut with SalI and HindIII and into which a modified subunit II (of Table VII) was inserted. The specific modifications of subunit II involved assembly with segment 39 altered to replace the alanine-specifying codon, GCT, with a threonine-specifying ACT codon and replace the isoleucine-specifying codon, ATT, with an ATG codon. Corresponding changes in complementary bases were made in section 40 of subunit LeIFN-FII.

The following Examples 9 and 10 relate to practice of the invention in the microbial synthesis of consensus human leukocyte interferon polypeptides which can be designated as analogs of human leukocyte interferon subtype F.

EXAMPLE 9

"Consensus human leukocyte interferon" ("IFN-Con," "LeIFN-Con") as employed herein shall mean a non-naturally-occurring polypeptide which predominantly includes those amino acid residues which are common to all naturally-occurring human leukocyte interferon subtype sequences and which includes, at one or more of those positions wherein there is no amino acid common to all subtypes, an amino acid which predominantly occurs at that position and in no event includes any amino acid residue which is not extant in that position in at least one naturally-occurring subtype. (For purposes of this definition, subtype A is positionally aligned with other subtypes and thus reveals a "missing" amino acid at position 44.) As so defined, a consensus human leukocyte interferon will ordinarily include all known common amino acid residues of all subtypes. It will be understood that the state of knowledge concerning naturally-occurring subtype sequences is continuously developing. New subtypes may be discovered which may destroy the "commonality" of a particular residue at a particular position. Polypeptides whose structures are predicted on the basis of a later-amended determination of commonality at one or more positions would remain within the definition because they would nonetheless predominantly include common amino acids and because those amino acids no longer held to be common would nonetheless quite

34

likely represent the predominant amino acid at the given positions. Failure of a polypeptide to include either a common or predominant amino acid at any given position would not remove the molecule from the definition so long as the residue at the position occurred in at least one subtype. Polypeptides lacking one or more internal or terminal residues of consensus human leukocyte interferon or including internal or terminal residues having no counterpart in any subtype would be considered analogs of human consensus leukocyte interferon.

Published predicted amino acid sequences for eight cDNA-derived human leukocyte interferon subtypes were analyzed in the context of the identities of amino acids within the sequence of 166 residues. See, generally, Goedell, et al., *Nature*, 290, pp. 20-26 (1981) comparing LeIFN-A through LeIFN-H and noting that only 79 amino acids appear in identical positions in all eight interferon forms and 99 amino acids appear in identical positions if the E subtype (deduced from a cDNA pseudogene) was ignored. Each of the remaining positions was analyzed for the relative frequency of occurrence of a given amino acid and, where a given amino acid appeared at the same position in at least five of the eight forms, it was designated as the predominant amino acid for that position. A "consensus" polypeptide sequence of 166 amino acids was plotted out and compared back to the eight individual sequences, resulting in the determination that LeIFN-F required few modifications from its "naturally-occurring" form to comply with the consensus sequence.

A program for construction of a manufactured IFN-Con DNA sequence was developed and is set out below in Table VIII. In the table, an asterisk designates the variations in IFN- α F needed to develop LeIFN-Con₁, i.e., to develop the [Arg²², Ala⁷⁶, Asp⁷⁸, Glu⁷⁹, Tyr⁸⁶, Tyr⁹⁰, Leu¹⁵⁶, Thr¹⁵⁶, Asn¹⁵⁷, Leu¹⁵⁸] analog of IFN- α F. The illustrated top strand sequence includes, wherever possible, codons noted to the subject of preferential expression in *E. coli*. The sequence also includes bases providing recognition sites for SalI, HindIII, and BstE2 at positions intermediate the sequence and for XbaI and BamHI at its ends. The latter sites are selected for use in incorporation of the sequence in a pBR322 vector, as was the case with the sequence developed for IFN- α F and its analogs.

```

      1   10
Met—Cys—Asp—Leu—Pro—Gln—Thr—His—Ser—Leu—Gly—Asn—Arg—Arg—
ATG TGT GAT TTA CCT CAA ACT CAT TCT CTT CGT AAC CGT CGC
      20
Ala—Leu—Ile—Leu—Leu—Ala—Glu—Met—Arg—Arg—Leu—Ser—Pro—Phe—
GCT CTG ATT CTG CTG GCA CAG ATG CGT CGT ATT TCC CCG TTT
      30
      40
Ser—Cys—Leu—Lys—Asp—Arg—His—Asp—Phe—Gly—Phe—Pro—Gln—Glu—
AGC TGC CTC AAA GAC CGT CAC GAC TTC GGC TTT CCG CAA GAA
      50
      60
Glu—Phe—Asp—Gly—Asn—Gln—Phe—Gln—Lys—Ala—Gln—Ala—Ile—Ser—
GAG TTC GAT GGC AAC CAA TTC CAG AAA OCT CAG GCA ATC TCT
      70
      80
Val—Leu—Ile—Glu—Met—Ile—Gln—Gln—Thr—Phe—Asn—Leu—Phe—Ser—
GTA CTG CAC GAA ATG ATC CAA CAG ACC TTC AAC CTC TTT TCC
      90
      100
Thr—Lys—Asp—Ser—Ser—Ala—Ala—Trp—Asp—Gln—Ser—Leu—Leu—Glu—
ACT AAA GAC AGC TCT GCT GCT TGG GAC GAA AGC TTG CTC GAG
      110
      120
Lys—Phe—Tyr—Thr—Glu—Ile—Tyr—Gln—Gln—Leu—Asn—Asp—Leu—Gln—
AAG TTC TAC ACT GAA CTC TAT CAG CAG CTG AAC GAC CTG GAA
      130
      140
Ala—Cys—Val—Ile—Gln—Gln—Val—Gly—Val—Glu—Glu—Thr—Pro—Leu—
GCA TGC GTA ATC CAG GAA CTT GGT GTA GAA GAG ACT CGC CTC
      150
      160
      170
      180
      190
      200
      210
      220
      230
      240
      250
      260
      270
      280
      290
      300
      310
      320
      330
      340
      350
      360
      370
      380
      390
      400
      410
      420
      430
      440
      450
      460
      470
      480
      490
      500
      510
      520
      530
      540
      550
      560
      570
      580
      590
      600
      610
      620
      630
      640
      650
      660
      670
      680
      690
      700
      710
      720
      730
      740
      750
      760
      770
      780
      790
      800
      810
      820
      830
      840
      850
      860
      870
      880
      890
      900
      910
      920
      930
      940
      950
      960
      970
      980
      990
      1000
      1010
      1020
      1030
      1040
      1050
      1060
      1070
      1080
      1090
      1100
      1110
      1120
      1130
      1140
      1150
      1160
      1170
      1180
      1190
      1200
      1210
      1220
      1230
      1240
      1250
      1260
      1270
      1280
      1290
      1300
      1310
      1320
      1330
      1340
      1350
      1360
      1370
      1380
      1390
      1400
      1410
      1420
      1430
      1440
      1450
      1460
      1470
      1480
      1490
      1500
      1510
      1520
      1530
      1540
      1550
      1560
      1570
      1580
      1590
      1600
      1610
      1620
      1630
      1640
      1650
      1660
      1670
      1680
      1690
      1700
      1710
      1720
      1730
      1740
      1750
      1760
      1770
      1780
      1790
      1800
      1810
      1820
      1830
      1840
      1850
      1860
      1870
      1880
      1890
      1900
      1910
      1920
      1930
      1940
      1950
      1960
      1970
      1980
      1990
      2000
      2010
      2020
      2030
      2040
      2050
      2060
      2070
      2080
      2090
      2100
      2110
      2120
      2130
      2140
      2150
      2160
      2170
      2180
      2190
      2200
      2210
      2220
      2230
      2240
      2250
      2260
      2270
      2280
      2290
      2300
      2310
      2320
      2330
      2340
      2350
      2360
      2370
      2380
      2390
      2400
      2410
      2420
      2430
      2440
      2450
      2460
      2470
      2480
      2490
      2500
      2510
      2520
      2530
      2540
      2550
      2560
      2570
      2580
      2590
      2600
      2610
      2620
      2630
      2640
      2650
      2660
      2670
      2680
      2690
      2700
      2710
      2720
      2730
      2740
      2750
      2760
      2770
      2780
      2790
      2800
      2810
      2820
      2830
      2840
      2850
      2860
      2870
      2880
      2890
      2900
      2910
      2920
      2930
      2940
      2950
      2960
      2970
      2980
      2990
      3000
      3010
      3020
      3030
      3040
      3050
      3060
      3070
      3080
      3090
      3100
      3110
      3120
      3130
      3140
      3150
      3160
      3170
      3180
      3190
      3200
      3210
      3220
      3230
      3240
      3250
      3260
      3270
      3280
      3290
      3300
      3310
      3320
      3330
      3340
      3350
      3360
      3370
      3380
      3390
      3400
      3410
      3420
      3430
      3440
      3450
      3460
      3470
      3480
      3490
      3500
      3510
      3520
      3530
      3540
      3550
      3560
      3570
      3580
      3590
      3600
      3610
      3620
      3630
      3640
      3650
      3660
      3670
      3680
      3690
      3700
      3710
      3720
      3730
      3740
      3750
      3760
      3770
      3780
      3790
      3800
      3810
      3820
      3830
      3840
      3850
      3860
      3870
      3880
      3890
      3900
      3910
      3920
      3930
      3940
      3950
      3960
      3970
      3980
      3990
      4000
      4010
      4020
      4030
      4040
      4050
      4060
      4070
      4080
      4090
      4100
      4110
      4120
      4130
      4140
      4150
      4160
      4170
      4180
      4190
      4200
      4210
      4220
      4230
      4240
      4250
      4260
      4270
      4280
      4290
      4300
      4310
      4320
      4330
      4340
      4350
      4360
      4370
      4380
      4390
      4400
      4410
      4420
      4430
      4440
      4450
      4460
      4470
      4480
      4490
      4500
      4510
      4520
      4530
      4540
      4550
      4560
      4570
      4580
      4590
      4600
      4610
      4620
      4630
      4640
      4650
      4660
      4670
      4680
      4690
      4700
      4710
      4720
      4730
      4740
      4750
      4760
      4770
      4780
      4790
      4800
      4810
      4820
      4830
      4840
      4850
      4860
      4870
      4880
      4890
      4900
      4910
      4920
      4930
      4940
      4950
      4960
      4970
      4980
      4990
      5000
      5010
      5020
      5030
      5040
      5050
      5060
      5070
      5080
      5090
      5100
      5110
      5120
      5130
      5140
      5150
      5160
      5170
      5180
      5190
      5200
      5210
      5220
      5230
      5240
      5250
      5260
      5270
      5280
      5290
      5300
      5310
      5320
      5330
      5340
      5350
      5360
      5370
      5380
      5390
      5400
      5410
      5420
      5430
      5440
      5450
      5460
      5470
      5480
      5490
      5500
      5510
      5520
      5530
      5540
      5550
      5560
      5570
      5580
      5590
      5600
      5610
      5620
      5630
      5640
      5650
      5660
      5670
      5680
      5690
      5700
      5710
      5720
      5730
      5740
      5750
      5760
      5770
      5780
      5790
      5800
      5810
      5820
      5830
      5840
      5850
      5860
      5870
      5880
      5890
      5900
      5910
      5920
      5930
      5940
      5950
      5960
      5970
      5980
      5990
      6000
      6010
      6020
      6030
      6040
      6050
      6060
      6070
      6080
      6090
      6100
      6110
      6120
      6130
      6140
      6150
      6160
      6170
      6180
      6190
      6200
      6210
      6220
      6230
      6240
      6250
      6260
      6270
      6280
      6290
      6300
      6310
      6320
      6330
      6340
      6350
      6360
      6370
      6380
      6390
      6400
      6410
      6420
      6430
      6440
      6450
      6460
      6470
      6480
      6490
      6500
      6510
      6520
      6530
      6540
      6550
      6560
      6570
      6580
      6590
      6600
      6610
      6620
      6630
      6640
      6650
      6660
      6670
      6680
      6690
      6700
      6710
      6720
      6730
      6740
      6750
      6760
      6770
      6780
      6790
      6800
      6810
      6820
      6830
      6840
      6850
      6860
      6870
      6880
      6890
      6900
      6910
      6920
      6930
      6940
      6950
      6960
      6970
      6980
      6990
      7000
      7010
      7020
      7030
      7040
      7050
      7060
      7070
      7080
      7090
      7100
      7110
      7120
      7130
      7140
      7150
      7160
      7170
      7180
      7190
      7200
      7210
      7220
      7230
      7240
      7250
      7260
      7270
      7280
      7290
      7300
      7310
      7320
      7330
      7340
      7350
      7360
      7370
      7380
      7390
      7400
      7410
      7420
      7430
      7440
      7450
      7460
      7470
      7480
      7490
      7500
      7510
      7520
      7530
      7540
      7550
      7560
      7570
      7580
      7590
      7600
      7610
      7620
      7630
      7640
      7650
      7660
      7670
      7680
      7690
      7700
      7710
      7720
      7730
      7740
      7750
      7760
      7770
      7780
      7790
      7800
      7810
      7820
      7830
      7840
      7850
      7860
      7870
      7880
      7890
      7900
      7910
      7920
      7930
      7940
      7950
      7960
      7970
      7980
      7990
      8000
      8010
      8020
      8030
      8040
      8050
      8060
      8070
      8080
      8090
      8100
      8110
      8120
      8130
      8140
      8150
      8160
      8170
      8180
      8190
      8200
      8210
      8220
      8230
      8240
      8250
      8260
      8270
      8280
      8290
      8300
      8310
      8320
      8330
      8340
      8350
      8360
      8370
      8380
      8390
      8400
      8410
      8420
      8430
      8440
      8450
      8460
      8470
      8480
      8490
      8500
      8510
      8520
      8530
      8540
      8550
      8560
      8570
      8580
      8590
      8600
      8610
      8620
      8630
      8640
      8650
      8660
      8670
      8680
      8690
      8700
      8710
      8720
      8730
      8740
      8750
      8760
      8770
      8780
      8790
      8800
      8810
      8820
      8830
      8840
      8850
      8860
      8870
      8880
      8890
      8900
      8910
      8920
      8930
      8940
      8950
      8960
      8970
      8980
      8990
      9000
      9010
      9020
      9030
      9040
      9050
      9060
      9070
      9080
      9090
      9100
      9110
      9120
      9130
      9140
      9150
      9160
      9170
      9180
      9190
      9200
      9210
      9220
      9230
      9240
      9250
      9260
      9270
      9280
      9290
      9300
      9310
      9320
      9330
      9340
      9350
      9360
      9370
      9380
      9390
      9400
      9410
      9420
      9430
      9440
      9450
      9460
      9470
      9480
      9490
      9500
      9510
      9520
      9530
      9540
      9550
      9560
      9570
      9580
      9590
      9600
      9610
      9620
      9630
      9640
      9650
      9660
      9670
      9680
      9690
      9700
      9710
      9720
      9730
      9740
      9750
      9760
      9770
      9780
      9790
      9800
      9810
      9820
      9830
      9840
      9850
      9860
      9870
      9880
      9890
      9900
      9910
      9920
      9930
      9940
      9950
      9960
      9970
      9980
      9990
      10000
      10010
      10020
      10030
      10040
      10050
      10060
      10070
      10080
      10090
      10100
      10110
      10120
      10130
      10140
      10150
      10160
      10170
      10180
      10190
      10200
      10210
      10220
      10230
      10240
      10250
      10260
      10270
      10280
      10290
      10300
      10310
      10320
      10330
      10340
      10350
      10360
      10370
      10380
      10390
      10400
      10410
      10420
      10430
      10440
      10450
      10460
      10470
      10480
      10490
      10500
      10510
      10520
      10530
      10540
      10550
      10560
      10570
      10580
      10590
      10600
      10610
      10620
      10630
      10640
      10650
      10660
      10670
      10680
      10690
      10700
      10710
      10720
      10730
      10740
      10750
      10760
      10770
      10780
      10790
      10800
      10810
      10820
      10830
      10840
      10850
      10860
      10870
      10880
      10890
      10900
      10910
      10920
      10930
      10940
      10950
      10960
      10970
      10980
      10990
      11000
      11010
      11020
      11030
      11040
      11050
      11060
      11070
      11080
      11090
      11100
      11110
      11120
      11130
      11140
      11150
      11160
      11170
      11180
      11190
      11200
      11210
      11220
      11230
      11240
      11250
      11260
      11270
      11280
      11290
      11300
      11310
      11320
      11330
      11340
      11350
      11360
      11370
      11380
      11390
      11400
      11410
      11420
      11430
      11440
      11450
      11460
      11470
      11480
      11490
      11500
      11510
      11520
      11530
      11540
      11550
      11560
      11570
      11580
      11590
      11600
      11610
      11620
      11630
      11640
      11650
      11660
      11670
      11680
      11690
      11700
      11710
      11720
      11730
      11740
      11750
      11760
      11770
      11780
      11790
      11800
      11810
      11820
      11830
      11840
      11850
      11860
      11870
      11880
      11890
      11900
      11910
      11920
      11930
      11940
      11950
      11960
      11970
      11980
      11990
      12000
      12010
      12020
      12030
      12040
      12050
      12060
      12070
      12080
      12090
      12100
      12110
      12120
      12130
      12140
      12150
      12160
      12170
      12180
      12190
      12200
      12210
      12220
      12230
      12240
      12250
      12260
      12270
      12280
      12290
      12300
      12310
      12320
      12330
      12340
      12350
      12360
      12370
      12380
      12390
      12400
      12410
      12420
      12430
      12440
      12450
      12460
      12470
      12480
      12490
      12500
      12510
      12520
      12530
      12540
      12550
      12560
      12570
      12580
      12590
      12600
      12610
      12620
      12630
      12640
      12650
      12660
      12670
      12680
      12690
      12700
      12710
      12720
      12730
      12740
      12750
      12760
      12770
      12780
      12790
      12800
      12810
      12820
      12830
      12840
      12850
      12860
      12870
      12880
      12890
      12900
      12910
      12920
      12930
      1294
```